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Review article

# Biological control of *Bemisia tabaci* with fungi ☆

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**Abstract**

Recent advances in production, formulation, and application of insect pathogenic fungi have resulted in improvements in long-standing whitefly mycoinsecticide products based on *Verticillium lecanii*, and development and registration of several new products based on *Paecilomyces fumosoroseus* and *Beauveria bassiana*. These products have the capacity to suppress and, in some instances, provide good control of whiteflies in both greenhouse and field crops. However, numerous factors continue to impede the commercial development of fungi as whitefly biological control agents. These include slow action, poor adulticidal activity, potentially negative interactions with commonly used fungicides, relatively high cost, limited shelf life, and dependence on favorable environmental conditions. Development of methods and strategies for overcoming these limitations has progressed, however, and various practices that enhance mycoinsecticide efficacy have been identified. Principal recommendations include: (1) initiating treatments against the early stages of the pest to prevent population buildup, (2) targeting pest populations developing under moderate environmental conditions (e.g., during spring or fall growing seasons), (3) selecting crops amenable to multiple, highly efficient spray applications, and (4) applying fungi asynchronously with incompatible fungicides. Commercial markets for these products have been slow to develop and remain unstable in the face of strong competition from less costly, highly efficacious chemical insecticides. Nevertheless, continuing problems with chemical insecticide resistance and environmental and food contamination support continued development of fungi as relevant tools in the whitefly biological control arsenal. 

*Keywords:* *Bemisia tabaci*; *Bemisia argentifolli*; Biological control; Fungi

**Contents**



☆ Recent evidence suggests that *B. tabaci* represents a species complex with numerous biotypes and two described cryptic species. The binomial *B. tabaci* here is used in the broadest sense to include all member of the species complex unless a more specific designation is indicated.

Use of product names is necessary to report factually on available data; however, the USDA and EMBRAPA neither guarantees nor warrants the standard of the product, and the use of the name by USDA/EMBRAPA implies no approval of the product to the exclusion of others that may also be suitable.

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## 1. Introduction

The potential for resistant *Bemisia tabaci* populations to develop as a consequence of intensive use of chemical insecticides has stimulated studies on integrated pest management strategies in which biological control may play a significant role. The importance of predators and parasitoids has been discussed elsewhere (Gerling, 1990; Heinz, 1996; Nordlund and Legaspi, 1996, Gerling et al., 2001; Naranjo, 2001). This paper will focus on the potential for microbial control.

Whiteflies feed by piercing the tissues of plants and sucking sap directly from the vascular bundles. Consequently, they are not susceptible to many common insect pathogens, including bacteria and viruses, that are normally transmitted via host feeding on contaminated foliage. Most entomopathogenic fungi, on the other hand, infect their hosts by direct penetration of the body wall. Surveys have revealed that they are among the most important natural enemies of whiteflies (reviewed by Lacey et al., 1996), and various species have been registered or are under development as microbial control agents.

Most reports of natural fungal infections of *Bemisia* spp. refer to species of Hyphomycetes, especially *Paecilomyces*, *Verticillium* and *Aschersonia* spp. (Table 1). Epizootics of *A. aleyrodis* have been reported from *B. tabaci* populations; however, most microbial control efforts with this fungus have targeted the greenhouse whitefly, *Trialeurodes vaporariorum* (Lacey et al., 1996). Accounts of Entomophthorales attacking *B. tabaci* are rare (Steinkraus et al., 1998), and these fungi have proven difficult to develop for microbial control applications. Fungi in the genera *Acremonium*, *Cladosporium*, *Aspergillus* and *Fusarium* may be found associated with whiteflies, but are usually saprophytic or opportunistic and will not be discussed here.

## 2. Natural occurrence of entomopathogenic fungi

Under certain conditions, natural epizootics of indigenous entomopathogenic fungi can suppress *B. tabaci* populations. For example, epizootics caused by *Paecilomyces fumosoroseus* can lead to substantial reductions in *B. tabaci* populations during or immediately following rainy seasons or even prolonged periods of cool, humid conditions in the field or greenhouse (Carruthers et al., 1993; Lacey et al., 1993; Castineiras, 1995). However, in general, epizootics of naturally occurring fungi cannot be relied upon for control. Only a few species of fungi have the capacity to cause high levels of mortality, and development of natural epizootics is not only dependent on the environmental conditions described above, but also strongly influenced by various crop production practices, making their

Table 1
Natural occurrence of entomopathogenic fungi on *Bemisia* populations[a]

| Fungus | Location | Source |
|---|---|---|
| Hyphomycetes | | |
| *Aschersonia aleyrodis* | Taiwan | Yen and Tsai (1969)[b] |
| | USA | Berger (1921)[c] |
| *Aschersonia andropogonis* | Taiwan | Yen and Tsai (1969)[b] |
| *Aschersonia cf. goldiana* | Brazil | Lourenção et al. (1999) |
| | Taiwan | Yen and Tsai (1969)[b] |
| *Beauveria bassiana* | Israel | Ben-Ze'ev et al. (1994) |
| *Paecilomyces farinosus* | Greece | Kirk et al. (1993) |
| | India | Nene (1973)[c] |
| *Paecilomyces fumosoroseus* | Brazil | Sosa-Gómez et al. (1997) |
| | Venezuela | R. Hall (pers. communication) |
| | Mexico | Garza Gonzalez (1993) |
| | Cuba | Castineiras (1995) |
| | Trinidad | Hall et al. (1994) |
| | Hawaii | R. Humber (pers. communication) |
| | USA | Carruthers et al. (1993) |
| | India | Balakrishnan and Nene (1980), Lacey et al. (1993) |
| | Nepal | Lacey et al. (1993) |
| | Pakistan | Lacey et al. (1993) |
| | Indonesia | Humber (1992) |
| | Philippines | T. Poprawski and R. Carruthers (pers. communication) |
| | Japan | S. Kurogi (pers. communication) |
| *Verticillium lecanii* | Colombia | Drummond et al. (1987) |
| | Venezuela | R. Hall (pers. communication) |
| | Mexico | Nier et al. (1991) |
| | Israel | Ben-Ze'ev (1993) |
| | Denmark | R. Humber (pers. communication) |
| | Spain | Lacey et al. (1993) |
| | Japan | S. Kurogi (pers. communication) |
| Entomophthorales | | |
| *Conidiobolus* spp. | Israel | Ben-Ze'ev (1993), Gindin and Ben-Ze'ev (1994) |
| | USA | R. Carruthers (pers. communication) |
| *Entomophthora* sp. | USA | R. Carruthers (pers. communication) |
| *Zoophthora (Erynia) radicans* | Chad | Silvie and Papierok (1991) |
| | Israel | Ben-Ze'ev et al. (1988) |
| Unidentified species | Brazil | Sosa-Gómez et al. (1997) |
| | USA | S. Wraight (unpublished observation) |

[a] Adapted/updated from Lacey et al. (1996).
[b] Mentioned by Fransen (1990).
[c] Mentioned by Cock (1993).

occurrence unpredictable. Also, epizootics often occur after intense injury has already been inflicted by whiteflies.

## 3. Use of fungi in inundative control strategies

In perennial crops, conditions usually support prolonged survivorship of natural enemies, enabling inoculative introductions to be successful (Dowell, 1990); however, the short cycles of most field crops do not allow natural enemy populations to become well established and increase to desirable levels. Consequently, development of biological control strategies for *B. tabaci* has focused, to a considerable degree, on inundative introductions of natural enemies including fungi. The most extensively researched and utilized approach for *B. tabaci* control with fungi relies on the frequent spraying of high doses of infective propagules (Wraight and Carruthers, 1999).

Fungal pathogens possess a demonstrated capacity to provide useful control of *B. tabaci* whiteflies under a broader range of conditions than was once commonly believed. Numerous laboratory and field studies have revealed that the high ambient humidity conditions required for development of natural epizootics are not necessarily required for fungal infection. Many pathogens find sufficient moisture for germination and host penetration within the leaf or insect microclimate boundary layer. This phenomenon has been demonstrated with respect to infection of whitefly nymphs by *Beauveria bassiana* and *P. fumosoroseus* (Wraight et al., 2000). Recent studies indicate that, in many instances, high temperature may be a more important factor limiting disease development than moisture (Inglis et al., 1997; Fargues et al., 1997b). This is especially significant with regards to control of *B. tabaci*, which is a key pest in regions with hot, dry climates. Unfortunately, the effects of temperature on efficacy of fungal biological control agents are difficult to characterize and remain poorly understood with respect to most pest-pathogen systems. Temperature has profound effects on the physiology and development of both the insect host and fungal pathogen (which simultaneously affect host susceptibility and pathogen virulence), and these effects are influenced, in turn, by factors related to the host-plant. Evapotranspiration, for example, can lower leaf-surface temperatures to levels substantially below ambient (Willmer, 1986). Ambient temperature readings also cannot be considered representative of the internal body temperatures of insects exposed to solar radiation. By basking, for example, insects are able to elevate body temperatures substantially above ambient and to levels that can inhibit mycosis (Carruthers et al., 1992).

Evaluations of fungal pathogens for potential control of whiteflies have focused on all life stages. Poor control of *B. tabaci* eggs has been observed following treatments with *B. bassiana* (Ramos et al., 2000), *P. fumosoroseus* (Lacey et al., 1999), *P. farinosus* (Negasi et al., 1998), *P. amoenoroseus* (Candido, 1999) and *Verticillium lecanii* (Meade and Byrne, 1991). Similar results have been recorded for the adults of *B. tabaci* subjected to *B. bassiana* and *P. fumosoroseus* treatments (Wraight et al., 2000); however, under favorable conditions, the latter pathogen clearly possesses epizootic potential against adults (Osborne and Landa, 1992; Carruthers et al., 1993; Lacey et al., 1993). The entomophthoralean *Zoophthora* sp. was found naturally infecting only *B. tabaci* adults, whereas other life stages were not attacked (Silvie and Papierok, 1991). Similarly, bioassays with *Conidiobolus coronatus* showed it to be ineffective against eggs and nymphs, but able to cause approximately 95% mortality of *B. tabaci* adults at low dosages (Gindin and Ben-Ze'ev, 1994).

In contrast, the nymphal stages of *B. tabaci* are highly susceptible to infection by a number of fungi, including *B. bassiana* (Eyal et al., 1994; Wraight et al., 1998; Ramos et al., 2000; Vicentini et al., 2001), *P. amoenoroseus* (Candido, 1999), *P. fumosoroseus* (Eyal et al., 1994; Vidal et al., 1997a; Wraight et al., 1998) and *V. lecanii* (Meade and Byrne, 1991).

Young instars of *B. tabaci* tend to be more susceptible to fungal infections than the 4th instar, as shown in studies with *B. bassiana* (S. Vicentini, M. Faria and M.R.V. Oliveira, unpublished observations) and *P. fumosoroseus* (Osborne et al., 1990). On the other hand, bioassays of *B. bassiana* strain GHA against the 2nd, 3rd, and 4th instars of *B. tabaci* reported by Wraight (1997) showed no direct relationship between instar and $LC_{50}$. Meade and Byrne (1991) reported that there was no differential susceptibility among 1st, 2nd, and 3rd instar *B. tabaci* treated with *V. lecanii*.

In situations in which *B. tabaci* is a vector of viruses, the use of biological control agents becomes very difficult. As previously related, entomopathogenic fungi often do not provide effective control of adult whiteflies, and transmission of plant viruses may persist even at extremely low population densities. In some crops, such as tomatoes, the presence of a single adult whitefly per plant is sufficient to cause 100% infection with geminiviruses (L. Hilje, pers. comm.). Another situation in which fungi may not be recommended is when the tolerance for presence of whiteflies is extremely low, as in many ornamentals. In poinsettia, the presence of more than 0.3–0.7 nymph per $cm^2$ is not accepted (Helgesen and Tauber, 1974).

### 3.1. Selection of isolates

Different bioassay methods using leaves as substrates for eggs and nymphs of *B. tabaci* have been established. For melons, root formation on leaf petioles immersed in tap water guarantees the turgidity and normal coloration for a period of 20–25 days. Besides that, average mortality of nymphs at 7 and 14 days in the control was 0% and 1.2%, respectively, with 94.4% of individuals reaching the adult stage at day 14, indicating a satisfactory condition of the substrate (Vicentini et al., 2001). Until recently, bioassays for assessment of fungi against aleyrodids were based on complex and laborious or excessively artificial techniques. In some cases, experiments were carried out using whole plants. In the method employed by Landa et al. (1994), nymphs were detached from leaves and kept on glass slides. An excellent bioassay technique based on rooting of bean leaves has been developed at the University of Vermont (Negasi et al., 1998). A similar method, based on rooting of cabbage leaves in specific nutrient solutions was recently published by Lacey et al. (1999).

Numerous *P. fumosoroseus* isolates, collected in various geographic regions (including Argentina, Brazil, France, India, Italy, Mexico, Nepal, Pakistan and the USA), have been assessed in the laboratory against nymphs of *B. tabaci*, and many of these have been identified as possessing microbial control potential (Landa et al., 1994; Hernandez Velazquez et al., 1995; Vidal et al., 1997a; Wraight et al., 1998). Twenty-nine isolates of diverse origins tested by Vidal et al. (1997a) produced mortality levels ranging from 68% to 94%. A narrow among-strain variability, in terms of virulence to the target insect, and lack of specificity toward the original hosts were observed. Similar results were reported by Wraight et al. (1998) with regards to *B. bassiana* isolates. On the other hand, Vicentini et al. (2001) screened 50 isolates of *B. bassiana* from diverse hosts against *B. tabaci* and found that 7 of the top 10 strains originated from Homoptera (Fig. 1). Isolates of *Metarhizium anisopliae* are also highly virulent toward nymphs of *B. tabaci* (Herrera et al., 1999).

In view of the aforementioned significance of temperature in determining fungal efficacy, it may be desirable, from the standpoint of biological control, to develop fungi with high-temperature tolerance. Studies have revealed that isolates of many common entomopathogenic Hyphomycetes exhibit a broad range of temperature optima (Fargues et al., 1997a; Ouedraogo et al., 1997; Vidal et al., 1997b).

### 3.2. Mass production and formulation

The improvement of mass production technologies continues to be of critical importance, although optimization of biphasic fermentation techniques providing high yields of stable conidia has been a major recent advance. Emerald BioAgriculture Corporation (formerly Mycotech) of Lansing, Michigan, USA, has an installed capacity for producing $5 \times 10^{18}$ conidia of *B. bassiana* per year, an amount sufficient for treatment of 200,000 ha at a rate of $2.5 \times 10^{13}$ conidia/ha (C. Bradley, pers. comm.). The average yield is $1 \times 10^{10}$ conidia/g of a proprietary substrate occupying approximately 1 l of fermentor space (Bradley et al., 1992; C. Bradley, pers. comm.). The selection of *B. bassiana* as the active ingredient in the mycoinsecticide products developed by Mycotech for whitefly control was strongly influenced by the greater conidia mass-production potential of this fungus relative to that of *P. fumosoroseus* (Wraight et al., 1998). Liquid-culture

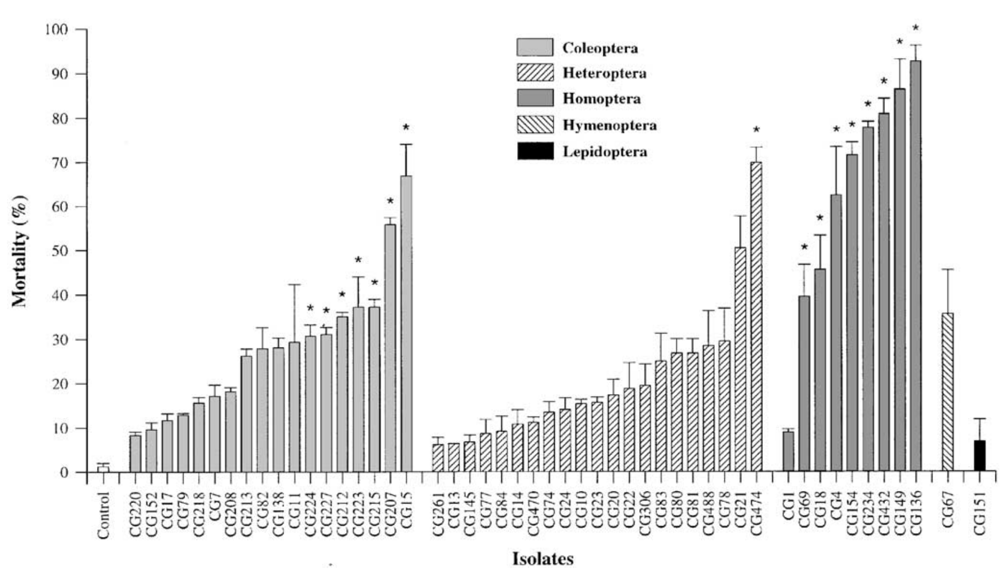


Fig. 1. Susceptibility of *B. tabaci* (biotype B) nymphs to *B. bassiana* strains isolated from different insect orders. Bars with * are significantly different from control ($P<0.05$).

production methods that produce high yields of desiccation-tolerant blastospores of *P. fumosoroseus* with cold-storage shelf life and high virulence against *Bemisia* whiteflies are being developed (Jackson et al., 1997; Vidal et al., 1998a).

The use of unformulated fungal products is generally not advisable, although many mycoinsecticides on the market fall into this category. Operational-scale amounts of unformulated fungal material can be extremely difficult to handle, especially dusty powders based on hydrophobic conidia. The performance of unformulated products may also be adversely affected by unfavorable environmental conditions, such as low relative humidity and high UV radiation. Formulated products, containing such materials as oils, humectants, UV-protectants, and nutrients to stimulate germination and growth, have great potential to provide better, more consistent results (Burges, 1998; Wraight et al., 2001). Oils, for example, improve adhesion and spreading of spores on the hydrophobic insect cuticle (Prior et al., 1988); spores may be carried by oil into microhabitats on the insect host or host plant where they are protected from wind, rain, solar radiation and other environmental stresses (Jenkins and Thomas, 1996; Ibrahim et al., 1999). Conidia of entomopathogenic fungi formulated in pure vegetable oil and adjuvant oils are claimed to be more resistant to UV radiation than unformulated conidia (Moore et al., 1993; Alves et al., 1998).

### 3.3. Delivery

Fungal pathogens possess a purely contact mode of action. Infectious propagules must be inoculated onto the target pest or onto substrates in the habitat from which secondary inoculation can be effected via pest movement or feeding. Under optimal environmental conditions, fungal hyphae may grow from germinated spores or fungus-killed hosts across leaf surfaces or other substrates to contact new hosts. Since whitefly nymphs are small, and because they do not browse or move about significantly on fungus-contaminated foliage, the most effective means of achieving rapid, high rates of infection of these insects under normal field environmental conditions is by direct inoculation. However, delivery of a lethal dose of fungal spores onto a large proportion of nymphs in a whitefly population using economically acceptable methods and numbers of spray applications is a difficult challenge. The most effective method of achieving direct hits on small targets on the undersides of leaves in a dense crop canopy is to direct the spray from below, atomize the spray into numerous, small droplets, and create sufficient air turbulence to penetrate into the canopy interior (Matthews, 1992; Bateman et al., 2000). Spray technologies capable of providing effective coverage of abaxial leaf surfaces in dense crop canopies have been available for many years. Good coverage is achievable with conventional hydraulic technologies, for example, by using drop-tubes with swivel-mounted nozzles, high pressures, and high spray volumes. Each of these requirements, however, comes at considerable cost. Use of drop tubes can require constant reconfiguration of a sprayer in order to make applications on crops with substantially different row spacing or architecture, or to make applications of various materials (e.g., herbicides vs. insecticides). High pressure, atomizing sprayers generate droplets that are highly susceptible to drift. Applications at high spray volumes (most easily achieved with conventional spray equipment by increasing pressure or reducing ground speed) are costly and time consuming. Research into developing technologies or sprayer configurations for achieving acceptable compromises with respect to these issues is being pursued (see Wraight and Carruthers, 1999). One approach has been to employ short, closely spaced drop tubes to carry nozzles just at or below the tops of the plants and directed forward and downward at a 45° angle to turn foliage (exposing the undersides) and to inject turbulent, atomized sprays directly into the canopy (Wraight and Carruthers, 1999). This configuration can be used regardless of row spacing, but still requires high volumes. New solenoid-controlled flow systems for applying atomized sprays at reduced volumes (Giles, 1997) may be a useful technology to apply to this problem. Air-blast or air-assisted spray technologies are also available that can be adjusted to direct airstreams at an advantageous spray angle (Hislop et al., 1993). Existing air-blast systems have been designed with the primary objective of delivering foliage-penetrating applications at low volumes (LV) or ultra-low volumes (ULV). Aerial ULV applications have been extensively researched and developed for applications of mycoinsecticides against large insects such as grasshoppers in sparsely vegetated rangelands (Bateman, 1992); however, this technology has not yet been adapted for widescale use in field crops. Additional research is needed to determine the potential efficacy of LV and ULV applications of fungal pathogens against field-crop pests. The importance of spray volume in terms not just of spray penetration and coverage, but also of droplet behavior upon host impact, is poorly understood. Higher volumes may be important for carrying fungal spores into cuticular folds or articulations on the host where the microenvironment is favorable for survival and infection.

Use of portable equipment can obviously improve targeting capabilities and thus efficacy (Wraight et al., 2000), but their use is economically constrained to small-areas (e.g., greenhouse or subsistence crops) or to low-technology crop production systems.

### 3.4. Compatibility with chemicals

In general, entomopathogenic fungi formulated for biological control are far less susceptible to the action of chemical insecticides than predatory and parasitic insects. Mycoinsecticides are compatible with a broad range of insecticides, allowing the establishment of sound integrated insect pest management practices. More serious issues arise, however, with respect to integration with plant disease management practices. Not surprisingly, numerous laboratory studies have shown that many common fungicides can be highly antagonistic toward beneficial insect fungi (reviewed by Roberts and Campbell, 1977; Glare and Milner, 1991). It is also evident from this work, however, that different species and strains of entomopathogenic fungi are highly variable in their susceptibility to these chemical antagonists, and that laboratory studies may not predict antagonistic effects under field conditions (Clark et al., 1982; Jaros-Su et al., 1999). It may be possible, in many cases, to devise strategies that minimize negative interactions. Some companies recommend that antagonistic chemicals be applied 2–3 days before or after applications of their fungus-based products. The utility of such a strategy was recently demonstrated in a field study in which efficacy of *B. bassiana* applied against Colorado potato beetle was not significantly affected by applications of fungicides for control of late blight (Jaros-Su et al., 1999).

### 3.5. Biosafety

This complex subject can be addressed only briefly in this short review. As a rule, insect fungi under development for whitefly control show no pathogenicity or toxicity toward vertebrates. To cite a few examples, the strain GHA of *B. bassiana* had no negative effect on fish (Collins et al., 1994) or birds (Althouse et al., 1997). Tests of the Apopka-97 strain of *P. fumosoroseus* have indicated no mutagenic or toxic effects against mammals (Sterk et al., 1996). There is much unpublished vertebrate safety data associated with product registrations. Safety tests related to registration of *Metarhizium* and *Beauveria* strains (including strain GHA) were reviewed by Goettel and Jaronski (1997).

Among the common whitefly pathogens, *Aschersonia* has a narrow insect host range, limited to whiteflies and coccids. In contrast, *Beauveria, Paecilomyces, Metarhizium* and *Verticillium* spp. have relatively broad arthropod host ranges. The great genetic variability within these species introduces additional levels of complexity with respect to host specificity. Not surprisingly, individual strains of fungal pathogens tend to have more restricted host ranges than the species to which they are assigned.

Entomopathogenic Hyphomycetes may have exceptionally broad insect host ranges under the ideal conditions of laboratory bioassays. However, actual impacts of fungus applications on field populations of nontarget invertebrates may be substantially less significant than might be predicted from laboratory studies. For example, the GHA strain of *B. bassiana* was virulent against the bee *Megachile rotundata* when applied at high dosages (Goerzen et al., 1990), but mortality levels in field experiments were <4% (Goettel and Johnson, 1994). Findings such as these underscore the need for much additional testing under natural conditions.

Thorough ecological evaluations are inherently difficult and time consuming, considering the large number and diversity of nontarget organisms that may be involved and their unpredictable levels of susceptibility to fungal pathogens. Strain GHA was highly infectious under laboratory conditions against larvae of the coccinellid beetle *Serangium parcesetosum*, an important whitefly predator, and the predation capacity of treated larvae was reduced by more than 50% (Poprawski et al., 1998). In contrast, however, a *Bemisia* isolate of *P. fumosoroseus* tested in the same study had no significant effects on *S. parcesetosum* larvae. Data reported by Jaronski et al. (1998) from a field test in cotton with unreplicated *B. bassiana* strain GHA treatments suggested a negative impact on populations of the predators *Orius* and *Nabis* spp., but not against Reduviid, Chrysopid and *Geocoris* spp.

Good compatibility between fungi and parasitoids has generally been observed in a number of laboratory, greenhouse, and field trials. For example, with respect to the *A. aleyrodis* and *Encarsia formosa* association, the latter did not oviposit on nymphs that were infected 7 days before or earlier (Fransen and van Lenteren, 1993). Conversely, spraying of the fungus 4 days after parasitization did not affect the survivorship and reproductive capacity of emerged parasitoids (Fransen and van Lenteren, 1994). It was observed that by day three postparasitization (after parasitoid egg hatch), *B. tabaci* nymphs were immune to infection by *B. bassiana* strain GHA (W.A. Jones and T.J. Poprawski, pers. comm.). Following releases of an *Eretmocerus* sp. at two field sites, Jaronski et al. (1998) observed no significant reductions in rates of parasitism as the result of *B. bassiana* strain GHA applications in melons. *P. fumosoroseus* was also reported to be very compatible with *Eretmocerus* sp. and the predator *Delphastus pusillus* (Osborne and Landa, 1992).

## 4. Commercial mycoinsecticide use

### 4.1. Protected crops

Greenhouses are subjected to very intense human intervention; however, a number of factors, including the potential for manipulating or stabilizing environmental conditions, the opportunity for regular sanitation, and the possibility of avoiding pest invasions while containing released natural enemies, are responsible for the success of biological control programs (van Lenteren and Woets, 1988; Dowell, 1990).

In the Netherlands, cultivation in greenhouses occupies only 0.5% of the agricultural area, but represents 17% of the agricultural value (van Lenteren, 1998). Tolerance for pests in such high-value crops is low creating a difficult challenge for biological control, but problems related to insecticide resistance and factors such as the demand of consumers for healthy foods have created favorable conditions for adoption of biological control in this and similar protected crop systems worldwide.

A number of mycoinsecticides are available for greenhouse use (Table 2). The product Mycotal, based on *V. lecanii*, is commercialized in Europe for control of *T. vaporariorum*, although it also has some activity against *B. tabaci*. Two to four applications made at 5–7 days intervals are normally recommended, each at a rate of 3 kg/ha ($3 \times 10^{13}$ conidia). The adjuvant oil Addit, an emulsified vegetable oil, used at 0.25% is claimed to improve the overall performance of the product (information provided by Koppert B.V.). According to Saito (1993), *V. lecanii* was as efficient as buprofezin in controlling nymphs of *B. tabaci* infesting tomatoes.

The fungus, *P. fumosoroseus* (strain Apopka-97), is also being used in greenhouses in several European countries for control of *T. vaporariorum* and *B. tabaci* and is also registered, but not yet commercialized, in the USA. Products containing this fungus (tradenamed PreFeRal in Europe and PFR-97 in the USA) are based on granular-formulated blastospores. These spores have inferior shelf life compared to conidia, but can be produced with greater efficiency. Most of the published efficacy data for these products relate to control of *T. vaporariorum*; PreFeRal was highly efficacious (>90% mortality) against this whitefly on greenhouse cucumbers and tomatoes with better efficacy on cucumbers (Bolckmans et al., 1995). In contrast, Vidal et al. (1998b) observed equal efficacy of PFR-97 applied against *Bemisia* infesting greenhouse cabbage, cucumbers, and three cultivars of tomato. Conidia-based products are commercially available in Latin America (e.g., Pae-sin in Mexico), and conidial preparations have also been developed in China (Fang et al., 1986) for whitefly control in both greenhouse and field crops.

Despite being rarely observed causing natural infection of whiteflies, the fungus *B. bassiana* shows great potential as a bioinsecticide. Different formulations, including a wettable powder and oil-based emulsifiable suspension based on strain GHA (originally collected in 1978 from a coleopteran), are marketed under the name BotaniGard in the USA, Mexico, and several Central American countries for control of whiteflies, aphids, thrips and mealybugs in greenhouses and nurseries.

In a greenhouse experiment with *Hibiscus*, the efficacy of *B. bassiana* (BotaniGard) under two humidity regimes was tested (T.X. Liu and P.A. Stansly, pers. comm.). High and low label dosages resulted in 80% and 92% control of *B. tabaci* nymphs when humidity was >95%. When humidity was reduced (65–75% during the day, >90% at night), control levels at the two doses (77% and 90%, respectively) were not significantly reduced. Mortality in untreated plots was near 4% in both cases.

### 4.2. Open field crops

Efficacy of mycoinsecticides under field conditions is generally not as good as in greenhouses, which explains the lower number of products for these agroecosystems when compared to protected agriculture (Table 2). This is attributable, in some cases, to harsh climatic condition, but more significantly, this problem is the result of economic constraints. The relatively low value of most field crops cannot support the more efficient application methods (e.g., hand targeted sprays) and high rates of application recommended for greenhouse crops. With respect specifically to whiteflies, poor efficacy against

Table 2
Mycoinsecticides available for *Bemisia tabaci* control

| Fungus | Product | Indication | Company | Country |
|---|---|---|---|---|
| *Beauveria bassiana* | BotaniGard | Greenhouse | Emerald BioAgriculture Corporation | USA |
| | Ago Biocontrol Beauveria | Greenhouse, field | Ago Biocontrol | Colombia |
| | Bea-Sin | Greenhouse, field | Agrobiológicos del Noroeste S.A. de C.V. | Mexico |
| | Boveril PM | Greenhouse | Itaforte BioProdutos | Brazil |
| *Paecilomyces fumosoroseus* | PreFeRal | Greenhouse | Thermo Trilogy/Biobest N.V. | Belgium |
| | Pae-Sin | Greenhouse, field | Agrobiológicos del Noroeste S.A. de C.V. | Mexico |
| *Verticillium lecanii* | Ago Biocontrol Verticillium | Greenhouse | Ago Biocontrol | Mexico |
| | Mycotal | Greenhouse, field | Koppert Biological Systems | Holland |

adults under normal weather conditions is another difficult problem. In the open field environment, crops are particularly vulnerable to mass migrations of whiteflies from surrounding vegetation or harvested fields.

Results at present do not allow a recommendation for general wide-scale use of mycoinsecticides for *B. tabaci* control in large hectarage, low value field crops. Recommendations can be offered, nonetheless, for some vegetable crops (especially cucurbits) that have been most extensively studied during recent years. Much of this work is based on field trials with the *B. bassiana* strain GHA products registered under the name Mycotrol.

In tests by Wraight et al. (2000), *P. fumosoroseus* and *B. bassiana* were applied against whitefly nymphs infesting cantaloupe and honeydew melons, cucumbers, and zucchini squash. Control levels of 86–98% were achieved with both pathogens following 3–5 applications of low to high rates of conidia ($1.25–5.0 \times 10^{13}$/ha) at 4–7 days intervals using a portable air-blast sprayer. *Beauveria* applications made with tractor-mounted air-blast and high-pressure hydraulic sprayers have been somewhat less efficacious producing maximum control of only 65–80% (Jaronski and Lord, 1996; Wraight and Bradley, 1996; Liu et al., 1999; Wraight and Carruthers, 1999).

There are very few published reports of tests in other vegetables. Poprawski (1999) reported 65% control of *B. tabaci* on collards after 5 weekly applications of *B. bassiana* strain GHA. Wraight et al. (1996) reported 75% control of whiteflies in broccoli from *P. fumosoroseus* compared to only 38% from *B. bassiana*.

Research applications in tomatoes and cotton have produced variable results. Wraight et al. (1996) reported good control of *B. tabaci* nymphs in fall-planted tomatoes sprayed with *B. bassiana* and *P. fumosoroseus* at the aforementioned high rate, using a portable air-blast sprayer. In southwestern Florida, on the other hand, Liu et al. (1999) observed no reductions in numbers of whitefly nymphs on tomatoes and eggplants treated with Mycotrol. These researchers, however, did note 40–50% infection of whitefly pupae. Akey and Henneberry (1998) compared the Naturalis-L (*B. bassiana* strain JW-1), Mycotrol, and PFR-97 in Arizona cotton; all were reported to significantly suppress populations of large *B. tabaci* nymphs. Jaronski et al. (1998) reported 80% control of whitefly nymphs following a single high-rate application in irrigated cotton in the Imperial Valley of California. However, in contrast to the above results, applications of Mycotrol by other investigators working in the Imperial Valley and in southern Texas observed no effective control of whiteflies in cotton (Wraight et al., 1996; Liu et al., 1999).

## 5. Mycoinsecticide-use recommendations

The list of factors that continue to impede the widespread adoption of mycoinsecticides for pest control is long and includes:

1. slow action (usually $>7$ days);
2. poor activity against adult whiteflies and need for frequent applications to control multiple, overlapping whitefly generations;
3. dependence upon favorable environmental conditions;
4. potential negative interactions with chemical fungicides applied for plant-disease control;
5. preference of whiteflies for the undersides of leaves, creating a difficult spray application (targeting) problem;
6. lack of adequate formulations necessary for the achievement of consistent control (especially for fungal propagules produced in liquid fermentations);
7. high costs due to difficulty of mass production of many fungal species or strains;
8. limited shelf life of most commercial products (especially at room temperature).

Research has been conducted, and is continuing, to identify application methods and strategies to overcome these constraints (see Wraight and Carruthers, 1999), and a number of recommendations can be offered.

1. Mycoinsecticides are best applied against low populations of first-instar nymphs, to prevent populations from building to uncontrollable levels. Fungal pathogens cannot be relied upon to control large populations of late-instar nymphs or adults, or any population under severe outbreak conditions. Hyphomycetes such as *B. bassiana* and *P. fumosoroseus* are generally compatible with a broad range of chemical insecticides needed for control of migrating adults that can rapidly overwhelm young seedlings and transmit plant viruses.
2. Advantage should be taken of favorable environmental conditions whenever possible, e.g., by targeting spring or fall crops, or by timing spray programs to coincide with predicted intervals of moderate temperature and high humidity in the field or greenhouse. High efficacy of *B. bassiana* and *P. fumosoroseus* was observed in cucurbit field tests during which average daily maximum temperatures did not exceed 32°C (Wraight et al., 2000).
3. Mycoinsecticides and incompatible fungicides should be applied asynchronously.
4. Users should select sprayers mechanically capable of targeting leaf undersides (e.g., hydraulic sprayers configured with drop tubes and swivel-mounted nozzles or air-blast or air-assisted sprayers with

adjustable air streams (especially to adjust the angle of the air stream relative to the ground)).

5. Whenever feasible, users should employ such methods as reducing ground speed and increasing spray pressure and volume to maximize spray coverage.
6. When possible, applications should be banded on the crop row to maximize dose at minimum cost. Banding can be especially advantageous in crops such as cucurbits planted on wide beds. In such cases, banded applications applied to small plants can employ a substantially greater rate of application (per treated area) at the same cost as a much lower broadcast rate (Vandenberg et al., 2000).
7. Considerations should be given to matching mycoinsecticide use to compatible crops and crop cultivars. For example, short-cycle crops such as pickle cucumbers are more easily protected than long-cycle crops that may support development of more whitefly generations. It is also easier to target whiteflies infesting plant varieties with limited vegetative growth (e.g., older melon varieties that produce few vines and sparse foliage versus new hybrids that produce dense, closed canopies). On the other hand, if good coverage can be achieved, advantage can be taken of favorable environmental conditions within dense crop canopies.
8. Mycoinsecticides should be stored under refrigeration whenever possible or under moderate temperatures (in air-conditioned storage facilities), if this is not practical. Recently, developed conidia-based formulations of some entomopathogenic Hyphomycetes can be stored at room temperature (25–30°) for at least 8 months (Jaronski, 1997; Jenkins et al., 1998; Alves, 1999).

## 6. Future research needs and conclusions

Much more research related to biological control is required to create a foundation for the rational use of natural enemies of *B. tabaci* within sustainable agroecosystems. Regarding mycoinsecticides, further improvements in technologies for mass production, stabilization, formulation, and delivery are of critical importance. Much additional compatibility testing with agrochemicals and other natural enemies is needed to support incorporation of these control agents into new and existing IPM systems.

Education of extension agents and growers for recognition of natural enemies and the necessity of constant monitoring must be given high priority, especially in developing countries. In order to do so, sampling methods must be established, and microbial control action thresholds developed for different pests and the various crops they attack.

Little is known regarding the potential for insect pest populations to develop resistance to fungal pathogens applied on a broad and intensive scale. It is encouraging that resistance is not reported to have developed as a consequence of the long-term use of *M. anisopliae* against sugarcane spittlebug in Brazil or of *B. bassiana* against pine caterpillar in China. Insect pathogenic fungi possess redundant enzyme systems conferring virulence in terms of capacity to degrade the insect integument (St. Leger, 1995). This redundancy obviously reduces the potential for development of resistance based on disruption of enzymes responsible for penetration of the host cuticle. On the other hand, the frequency and heritability of other resistance factors (e.g., chemical constituents of the host cuticle with antifungal properties) and the capacity of insect fungi to overcome these factors are essentially unknown. Estimating the potential for development of mycoinsecticide resistance and establishing recommendations for resistance management will require extensive fundamental and applied research.

It cannot be disputed that mycoinsecticides are at a clear disadvantage when compared to chemical insecticides solely on the basis of immediate to short-term crop-production economics. Chemicals exhibit extraordinary levels of efficacy and efficiency due in large part to systemic, vapor, or translaminar action. They are easy to apply, fast-acting, widely available, and storable for long periods under warehouse conditions. This combination of traits makes chemical insecticides extremely cost-effective compared to microbial control agents. Most would acknowledge that the 60–75% control currently achievable with mycoinsecticides in the field represents a significant and useful level of whitefly suppression, but this level of control is simply not competitive with novel insecticides capable of providing essentially 100% control at equal or significantly less cost. We contend, however, that in spite of these constraints, mycoinsecticides can be quite relevant in whitefly control. In particular, strategic use of these agents in well-conceived IPM programs could reduce the likelihood of pest populations developing resistance to chemical molecules. In fact, the replacement of chemical applications by use of fungal pathogens has potential to extend the market life of many chemical insecticides, and in that sense, they should be considered allies rather than competitors of the chemical-insecticide industry.

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